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AGGREGATION PHEROMONE OF *Carpophilus antiquus* (COLEOPTERA: NITIDULIDAE) AND KAIROMONAL USE OF *C. lugubris* PHEROMONE BY *C. antiquus*

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Abstract—Males of *Carpophilus antiquus* Melsheimer (Coleoptera: Nitidulidae) emit an aggregation pheromone that was found to be a novel hydrocarbon, (3*E*,5*E*,7*E*,9*E*)-6,8-diethyl-4-methyl-3,5,7,9-dodecatetraene. A synthetic scheme and spectra (mass and proton NMR) are given for the compound. Beetles produced the pheromone when feeding on a variety of media, including the brewer's yeast-based artificial diet, fermenting whole-wheat bread dough, corn, and prunes; live baker's yeast was generally added to the food media. Males held individually produced, on average, 25× more pheromone per beetle than males held in groups of 10 or more. Pheromone was not produced until males were at least 5 days old but was still detected from the oldest beetles tested (47 days). In field tests, the pheromone was attractive to both sexes of *C. antiquus*, and it was synergized by food volatiles: A combination of pheromone and fermenting whole wheat dough attracted 2.5× more beetles than pheromone alone, but dough by itself was not significantly more attractive than the control. Semiochemical interactions were studied among *C. antiquus* and two other sympatric species for which pheromones are known, *C. lugubris* Murray and *C. freemani* Dobson. *C. antiquus* responded readily to the pheromone of *C. lugubris*, but all other interspecific responses to the pheromones were weak. In a sample of naturally infested corn ears, the presence of *C. antiquus* was strongly associated with the presence of *C. lugubris*, as would be expected if the pheromone of *C. lugubris* serves as a kairomone for *C. antiquus*.

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Key Words—*Carpophilus antiquus*, *C. lugubris*, *C. freemani*, Coleoptera, Nitidulidae, pheromone, kairomone, hydrocarbon, tetraene.

INTRODUCTION

Carpophilus antiquus Melsheimer (Coleoptera: Nitidulidae) is a small (2.4 mm length), reddish brown sap beetle that is most often found associated with corn. Williams et al. (1983) and references therein reported that its range in North America is from New Jersey south through the Carolinas and westward into Minnesota and Missouri. We have found it to be quite common in corn fields and woodlands in central Illinois.

Male-produced aggregation pheromones have been identified for a number of sympatric species including *C. lugubris* Murray (Bartelt et al., 1991) and *C. freemani* Dobson (Bartelt et al., 1990b). We had previously noticed that *C. antiquus* responds clearly to the pheromone of *C. lugubris* (unpublished), and we wished to know whether the two species simply shared a pheromone or whether a more complex semiochemical interaction existed between them. We sought to clarify the relationship by identifying the pheromone produced by *C. antiquus*, by measuring the relative attractiveness of the species' pheromones in field experiments, and by determining the degree of association between species in naturally infested corn ears. The field experiments were expanded to include the pheromone of *C. freemani* also, when it became clear that this species was common enough to provide meaningful comparisons with the others.

Irrespective of ecological interactions among species, having a pheromone for *C. antiquus* could be of practical importance because the species is a significant member of the sap beetle complex attacking corn in the Midwest. Additional control and monitoring techniques for these beetles may become more valuable because they are now known to vector mycotoxin-producing fungi (Lussenhop and Wicklow, 1990).

METHODS AND MATERIALS

Beetles. A culture of *C. antiquus* was started from beetles collected near Kilbourne, Illinois, in April 1991. The rearing method followed the concepts described by Dowd and Weber (1991) except that the pinto beans in the diet were replaced by additional brewer's yeast (the "brewer's-yeast diet"). Later on, finely chopped pitted prunes were added to the diet as well (30/liter, the "prune–brewer's-yeast diet"). We found *C. antiquus* to be difficult to rear in large numbers, primarily because of poor egg production. However, best results were with the prune–brewer's-yeast diet. The culture eventually produced enough

beetles to allow pheromone identification, but beetle numbers were never sufficient for conducting wind-tunnel bioassays.

Pheromone Identification. Because laboratory bioassays could not be used to guide pheromone purification, the approach was to seek consistent, sex-specific differences in volatile collections from male and female beetles by gas chromatography (GC). Any sex-specific compounds would be considered as pheromone candidates, based on previous experience, and identification of these through spectroscopy and synthesis would be attempted. Pheromonal activity of the synthetic compounds would then be verified in field bioassays.

Pheromone Collections. Volatile collections from feeding beetles were made as described previously for *C. hemipterus* (Bartelt et al., 1990a). Briefly, male or female beetles and food materials were placed into 50-ml flasks. Filters of Tenax or, later, Super Q porous polymer (Alltech Associates, Deerfield, Illinois, for both materials) were used to clean the incoming air and to capture the volatiles from the feeding beetles. The temperature during collections was 27°C, the humidity of incoming air was ca. 30%, and the photoperiod in the incubator was 14:10 hr (light–dark). Volatiles were eluted from filters with hexane.

Several food types were tried: brewer's yeast diet, prune–brewer's-yeast diet, water-soaked corn seeds (autoclaved to retard mold formation), prunes, and whole-wheat bread dough (whole-wheat flour, sugar, and water in a 4:1:2 blend, by volume). Dried baker's yeast (Fleischman's Yeast Inc., Oakland, California) was sprinkled over all these media. In addition, the brewer's yeast and prune–brewer's-yeast diets were tried without additional baker's yeast. This range of food materials was chosen based on previous experience with other *Carpophilus* species (Bartelt et al., 1990a,b, 1991, and other, unpublished data). Live baker's yeast was usually added because nitidulid beetles are typically attracted to fermenting media, and they presumably colonize and emit pheromone readily from such food sources. Initially, sand (ca. 1-cm layer, extracted with hexane prior to use to reduce background volatiles) was placed in the bottoms of the flasks to provide a more natural feeding site for the beetles (i.e., the food–sand interface) and to retain dampness. Damp sand led to more rapid spoilage of the food, however, and this practice was discontinued.

Beetles were always segregated by sex, and between 1 and 150 individuals were added to flasks. The initial number was 150; it was expected that the pheromone would be more prominent against the background of food volatiles if more individuals were present. However, when fewer beetles were in the flasks, pheromone production per beetle was greater (see Results). Later collections were made from smaller numbers of beetles.

One hundred thirty-one male-derived and 56 female-derived volatile collections were analyzed. (Collections from females were terminated after it was clear that a male-specific compound existed). Multiple regression analysis was

used to explore relationships between pheromone production in males and factors such as beetle age, numbers of beetles in volatile collectors, and food source.

Chromatography and Spectroscopy. Instrumentation was as described previously (Bartelt et al., 1990a). Male- and female-derived volatile collections were compared by GC on a 15-m × 0.25-mm-ID capillary column (DB-1, with 1.0-μm film thickness, J&W Scientific, Folsom, California). Oven temperature was programmed from 70 to 220°C at 10°/min.

The male-derived samples for all days for which a male–female difference was detected were combined for further analysis. A parallel sample was prepared from the female-derived volatile collections. These were subjected to column chromatography on silica gel; the elution solvents were hexane and 5%, 10%, and 50% ether in hexane. These fractions were again analyzed by GC for male–female differences.

The major male-specific compound in the hydrocarbon fraction was analyzed by GC–mass spectrometry (GC-MS) and was further purified by high-performance liquid chromatography (HPLC) on a silver-nitrate-coated silica column (Heath and Sonnet, 1980); the solvent was 10% toluene in hexane. A nuclear magnetic resonance (NMR) proton spectrum was obtained for the purified compound (300 MHz, deuterobenzene).

Synthetic Hydrocarbons. The structures of five hydrocarbons discussed in the text are shown in Figure 1, along with assigned structure numbers. These are: (3*E*,5*E*,7*E*,9*E*)-6,8-diethyl-4-methyl-3,5,7,9-dodecatetraene **(1),** (2*E*,4*E*,6*E*,8*E*)-7-ethyl-3,5-dimethyl-2,4,6,8-undecatetraene **(5),** (2*E*,4*E*,6*E*,8*E*)-3,5,7-trimethyl-2,4,6,8-undecatetraene **(6),** (2*E*,4*E*,6*E*)-5-ethyl-3-methyl-2,4,6-nonatriene **(7),** and (3*E*,5*E*,7*E*)-6-ethyl-4-methyl-3,5,7-decatriene **(8).** The pheromone components of *C. lugubris* and *C. freemani* **(5–8)** were available from earlier research (Bartelt et al., 1990c, 1991).

The *C. antiquus* pheromone **(1)** was synthesized as outlined in Figure 1. Commercially available aldehyde **2** was used in a Wittig-Horner condensation with triethyl 2-phosphonobutyrate. The resulting ethyl ester was reduced to the corresponding alcohol with $LiAlH_4$, and the alcohol was converted to aldehyde **3** with MnO_2. These steps were repeated a second time, starting with **3** to yield aldehyde **4,** and a Wittig condensation using (propyl)triphenylphosphonium iodide completed tetraene **1.** Reaction conditions were as described previously for related syntheses (Bartelt et al., 1990c). The final product **1** and the ester intermediates were distilled (Kugelrohr). Compound **1** was passed through a silica gel column with hexane to remove polar impurities. Over the seven synthetic steps, the yield of all-*E* **1** was 8% from aldehyde **2,** and the purity of **1** in the final product was 61%. Impurities were primarily *Z* isomers of **1.** Another impurity (6%) was triene **8,** which was generated because of incomplete Wittig-Horner condensations. The product was diluted with hexane to 150 μg of **1** per microliter and was used to prepare trap baits for field tests. Chromatographic

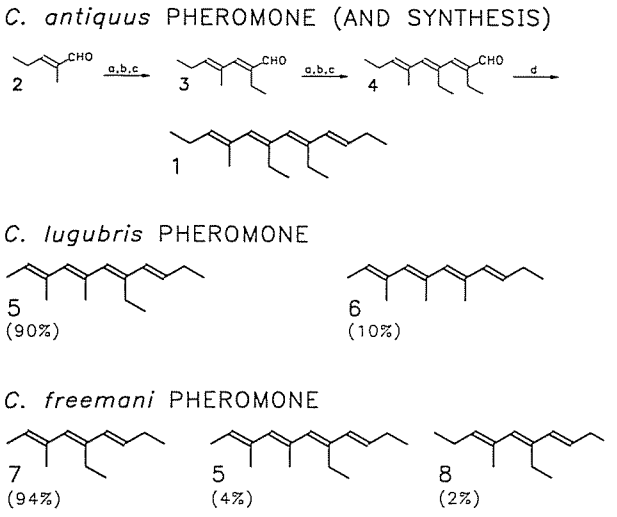

FIG. 1. Hydrocarbon pheromones of three *Carpophilus* species and assigned structure numbers. Synthetic scheme shown for *C. antiquus* pheromone **(1)**; a = Wittig-Horner condensation with triethyl 2-phosphonobutyrate, b = reduction with $LiAlH_4$, c = conversion to aldehyde with MnO_2, d = Wittig condensation with (propyl)triphenylphosphonium iodide (see text). For *C. lugubris* and *C. freemani*, proportions of components in pheromones are indicated as percentages.

removal of unwanted geometrical isomers and other impurities was not practical because of the large amount of material needed for field tests. An analytical sample of **1** was prepared by HPLC on the silver nitrate column (5% toluene in hexane) followed by HPLC on a size-exclusion column (PLgel 50A, Polymer Laboratories, Shropshire, U.K.) with hexane as solvent to remove the last traces of toluene prior to NMR analysis. As for the beetle-derived sample, a proton spectrum was obtained at 300 MHz in deuterobenzene.

Formulations for Field Use. Synthetic **1** was applied to rubber septa (20 mm × 11 mm diameter, red rubber, Aldrich Chemical Co., Milwaukee, Wisconsin) for use in field traps. The compound was loaded at the rate of 1.4 mg/septum and was immediately followed with 300 μl of methylene chloride. The release rate from the septa was 0.60 μg/hr during the first day after preparation, measured in volatile collections taken at 27°C and with an airspeed of 48 cm/min.

For *C. lugubris*, septum loads for **5** and **6** (0.44 and 0.05 mg, respectively) were chosen so that the emission rate of **5** was the same as that for **1** from the *C. antiquus* septa. (It was verified by GC that the *C. lugubris* pheromone used in the field study did not contain **1**). The load rates of **7** and **5** for the *C. freemani*

septa were 0.49 and 0.01 mg, respectively (the standard load rate in earlier studies). As discussed below, **8** was not added to the septa.

Field Studies. Experiments were conducted in 1992 near Kilbourne, Illinois, in an oak woods adjacent to a corn field, and near Washington, Illinois, in a stand of mixed hardwoods adjacent to an apple orchard. Wind-oriented funnel traps (Dowd et al., 1992) were used for these studies. The traps were hung from tree branches between 1 and 2 m above the ground, and traps were separated by at least 10 m. Within each block, traps were rerandomized weekly. Trap baits included the pheromone-treated septa described above and a source of food volatiles, which was fermenting whole wheat bread dough (ca. 15 g/trap).

One field study tested the activity of synthetic **1** and whether there was a synergistic response to synthetic **1** and food volatiles. Treatments were: **1** alone, dough alone, **1** plus dough, and empty (control) trap. All four treatments were present in each of two blocks. The beetles were collected weekly from September 15 to October 27 and were sorted according to species; captured *C. antiquus* were sexed. Both pheromone and dough baits were replaced weekly. This study was conducted only at Kilbourne.

A second study tested for cross-attraction between *C. antiquus* and *C. lugubris*. Treatments were **1** plus dough, *C. lugubris* pheromone (**5** + **6**) plus dough, and dough alone. Beetles were collected weekly and sorted according to species. Captured *C. antiquus* were sexed. This was conducted both at Kilbourne (four blocks) and at Washington (two blocks). This study ran from April 10 to May 15 and again from September 15 to October 27 at both locations. (In previous unpublished studies, *C. antiquus* never responded to traps in midsummer.) During the spring period, the pheromone baits were replaced every two weeks. Otherwise, all pheromone and dough baits were replaced weekly.

A fourth treatment, *C. freemani* pheromone (**7** + **5**) plus dough, was added to the experiment at Kilbourne between October 6 and October 27 because *C. freemani* were being captured in some of the other treatments in fairly high numbers.

The studies were analyzed as randomized complete block experiments by analysis of variance. Trap catches were transformed to the log $(X + 1)$ scale to stabilize variance.

Corn Ear Samples. As part of another study by one of us (P.F.D.), 120 milk-stage ears of corn were harvested on August 5, 1992, from a farm near Green Valley, Illinois. The corn was an open husk variety, which allowed beetles easy access to kernels. Ears were examined for *Carpophilus* beetles, and numbers of each species on each ear were recorded. A chi-square procedure was used to test for any association between the presence or absence of *C. antiquus* and *C. lugubris*.

RESULTS

Male-Specific Compound. By GC, one compound was detected in 101 of 131 male-derived volatile collections that was never detected in female-derived samples. Relative to *n*-alkanes, the compound had an equivalent chain length of 16.20 carbon units. After column chromatography on silica gel, the compound was present only in the hydrocarbon fraction. (No other sex-specific compounds were noted when the more polar silica gel fractions were compared.)

The molecular weight of the male-specific hydrocarbon was 232 by GC-MS, and the spectrum was identical to that shown in Figure 2. By analogy to the previously identified *Carpophilus* pheromones, the compound was postulated to be a 17-carbon tetraene; a molecular formula of $C_{17}H_{28}$ would account for the molecular weight of 232. When the male-derived, hydrocarbon fraction was purified further by HPLC on the silver nitrate column, the compound eluted 5.4–5.8 ml after injection (the void volume was 3.2 ml); elution after the solvent front supported the presence of double bond(s). After HPLC, a 12-μg sample of the unknown compound was available for NMR; by GC, the purity was 93%.

The NMR spectrum for the isolated compound was virtually identical to that for the synthetic tetraene (Figure 2) except for additional signals due to residual toluene from the HPLC step (δ 2.15 and >7) and unknown impurities (small singlets at δ 2.36, 1.95, 1.58, and 1.38). The spectrum indicated four ethyl groups and one methyl group attached to olefinic systems and five olefinic protons, for a total of 28 hydrogens. Two of the ethyl-group methylenes (δ 2.41 and 2.60) were split only by terminal methyls (δ 1.11 and 1.22, respectively, J = 7.5 Hz), but each of the other two (δ 2.09 and 2.11) was split by an olefinic proton (δ 5.56, J = 7.2 and δ 5.76, J = 6.6 Hz, respectively) as well as by terminal methyls (δ 0.99 and 1.02, respectively, J = 7.5 Hz). These two methylene quintets partially overlapped, giving the appearance of a four-proton sextet, and this signal was further complicated by the toluene signal in the natural sample. However, the triplet olefinic signals at δ 5.56 and 5.76 clearly indicated the attachment of two separate methylenes. The signal at δ 5.76 was further split by the proton at δ 6.13. The large coupling constant (J = 15.7 Hz) was consistent with an *E* configuration at a disubstituted double bond. A one-proton singlet (δ 6.10) overlapped the right-hand peak of the one-proton doublet centered at δ 6.13. There was another unsplit olefinic proton at δ 5.95 and an unsplit methyl signal at δ 1.78.

The shifts and couplings were matched to portions of similar structures reported previously (Bartelt et al., 1991, 1992), and compound **1** was chosen for synthesis as the probable structure of the pheromone. Synthetic **1** matched the natural compound by GC, NMR, and mass spectrometry.

Minor amounts (<5% as abundant as **1**) of other male-specific compounds were also detected when the combined, beetle-derived hydrocarbon fractions

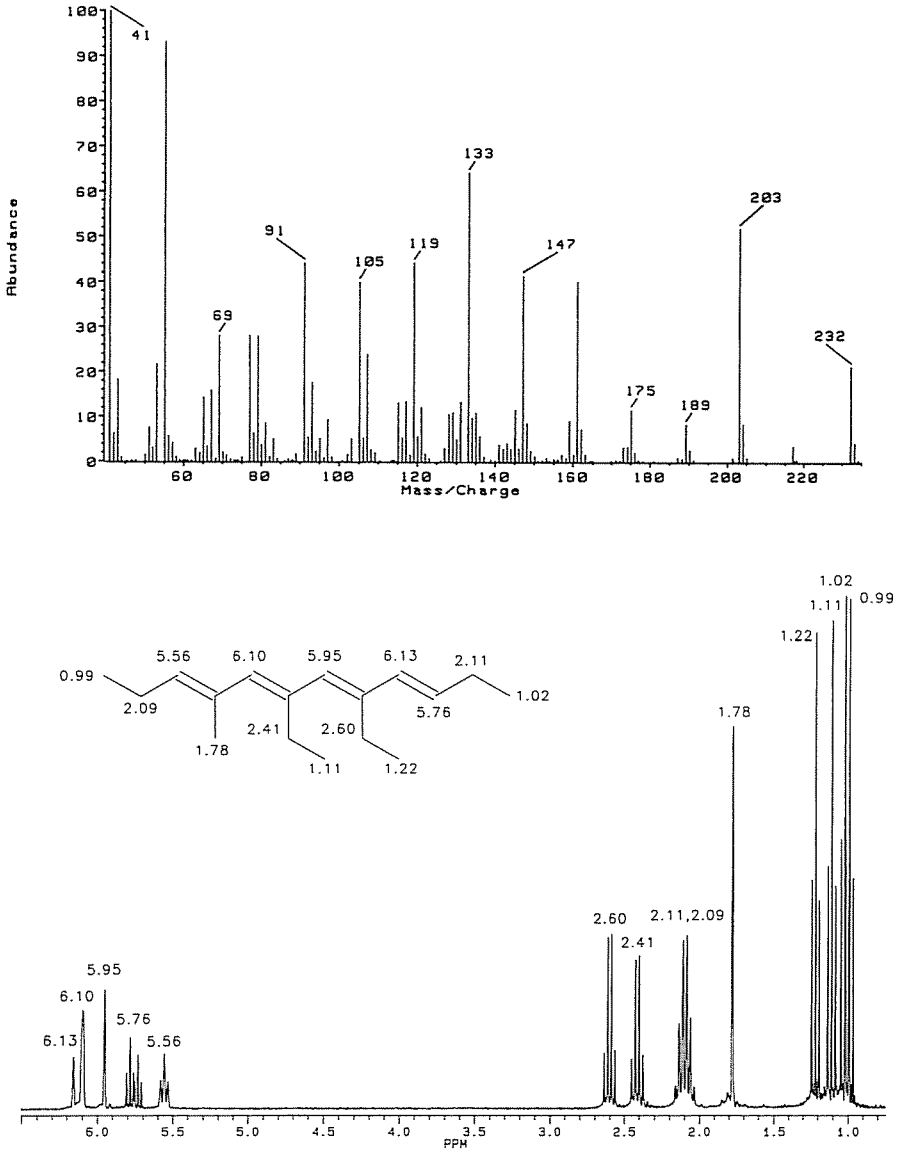

FIG. 2. Electron impact mass spectrum and proton NMR spectrum for synthetic **1.**

were carefully compared by GC. By GC retention (elution before **1**), mass spectrometry (spectra similar to **1**), and experience with other tetraenes (Bartelt et al., 1992), these other compounds were believed to be *Z* isomers of **1.** Some isomerization/degradation has invariably occurred with all of the tetraenes (Bartelt et al., 1992), and it is unknown whether the minor isomers are actually in emissions from *C. antiquus*. These were not analyzed further in this study.

Pheromone Production. Pheromone production in the laboratory was highly variable, but it was clearly dependent on beetle age and on numbers of beetles per collection flask (Table 1). By regression analysis, old beetles (≥ 5 days) produced significantly more pheromone than young beetles (1–4 days old, $P < 0.001$). Furthermore, on a per male basis, collections from single males contained significantly more pheromone than those from groups of 10 or more males ($P < 0.001$). The interaction of these factors was also significant ($P < 0.001$) because the difference between single and crowded beetles was seen only for old beetles; young beetles did not produce pheromone at any density. The highest observed production rate for a single male was 279 ng/day at 17 days of age. The largest value for a group was 31 ng/male/day for thirty 17-day-old males. Pheromone production could continue for over a month; one group of 50 males produced 3.6 ng/male/day at age 39 days and 0.5 ng/male/day at 47 days.

Pheromone production was detected for all of the food materials tested. The highest rates of pheromone production occurred for beetles feeding on the prune–brewer's-yeast diet with added baker's yeast, but differences among food media were not significant. Pheromone emission ceased whenever the collection flask became too wet (condensation on flask wall) or too dry (food material hard and brittle). Pheromone was detected in flasks containing some mold, but mold

TABLE 1. PHEROMONE PRODUCTION BY MALE *C. antiquus* (FITTED VALUES FROM REGRESSION ANALYSIS[a] OF 131 VOLATILE COLLECTIONS)

Beetles in collector		Pheromone production	Range	Number of
Age (days)	Number	(ng/male/day)	(ng/male/day)	collections
1–4	1	0.0		6
1–4	≥10	0.1	0–0.3	12
≥5	1	102	0–279	36
≥5	≥10	4.1	0–31	77

[a] For regression analysis, $R^2 = 0.46$, $P < 0.001$. Difference between age categories, difference between beetle-number categories, and the age by beetle-number interaction were all significant ($P < 0.001$).

on all food surfaces was an unhealthy situation that usually led to beetle mortality.

Activity of C. antiquus *Pheromone.* Synthetic **1** was attractive to *C. antiquus* in the field (Table 2). Fermenting dough was not significantly more attractive than the control. Dough did synergize the pheromone, however, with the combination attracting 2.5× more *C. antiquus* than the pheromone alone. Both sexes responded about equally to the pheromone treatments. *C. lugubris* was not affected by the pheromone of *C. antiquus*; both treatments containing dough attracted slightly more beetles than those that did not. *C. freemani* responded slightly but significantly to the combination of **1** and dough; the other treatments were not different from the control.

Cross-attraction. In all field tests, *C. antiquus* responded to the pheromone of *C. lugubris* plus dough dramatically better than to dough but generally not as well as to its own pheromone plus dough (Table 3). On the other hand, *C. lugubris* responded well only to its own pheromone plus dough. Combinations of the other pheromones with dough were not more effective for *C. lugubris* than dough by itself. Finally, *C. freemani* did respond significantly to the *C. antiquus* bait plus dough, but this effect was very small in comparison with the response to its own pheromone. No significant cross attraction between *C. lugubris* and *C. freemani* was observed in this study.

Corn Ear Infestation. The distributions of *C. antiquus* and *C. lugubris* were highly correlated in the corn ear sample (Table 4). Of the 27 ears on which *C. antiquus* was found, 25 also had *C. lugubris*. This was significantly higher than the 13.5 ears expected under the null hypothesis of independent distributions of the species (Pearson's chi-square statistic = 25.3, 1 *df*, $P << 0.001$).

TABLE 2. ATTRACTIVENESS OF SYNTHETIC *C. antiquus* PHEROMONE (COMPOUND **1**) TO THREE *Carpophilus* SPECIES UNDER FIELD CONDITIONS, (SEPTEMBER 15–OCTOBER 27, KILBOURNE, ILLINOIS

Treatment	Mean trap catch per week[a]		
	C. antiquus (% males)	*C. lugubris*	*C. freemani*
Compound **1** + dough	53.7 a (55%)	2.6 a	2.0 a
Compound **1**	18.8 b (55%)	0.0 b	0.3 b
Dough	0.1 c	2.9 a	0.0 b
Control (empty trap)	0.0 c	0.0 b	0.0 b

[a] In each column, means followed by the same letter are not significantly different (LSD, 0.05 level). Analysis was done in log (X + 1) scale, and means were returned to numerical scale for presentation. N = 12 for all means. Sex of captured *C. antiquus* shown for total captures over 10.

TABLE 3. ATTRACTIVENESS OF THREE SYNTHETIC *Carpophilus* PHEROMONES TO THREE SPECIES UNDER FIELD CONDITIONS[a]

Pheromone treatment	Mean trap catch per week[b]		
	C. antiquus (% males)	*C. lugubris*	*C. freemani*
April 10–May 15 (N = 30)			
C. antiquus + dough	92.4 a (46%)	1.1 b	0.5 a
C. lugubris + dough	17.3 b (47%)	88.6 a	0.1 b
Dough	0.5 c (52%)	1.5 b	0.0 b
September 15–October 27 (N = 24)			
C. antiquus + dough	17.8 a (55%)	1.3 b	2.1 a
C. lugubris + dough	4.8 b (53%)	78.0 a	0.3 b
Dough	0.1 c	1.4 b	0.0 b
October 6–27 (N = 6) (Kilbourne site only)			
C. antiquus + dough	59.1 a (52%)	0.6 b	1.4 b
C. lugubris + dough	34.4 a (51%)	47.9 a	1.3 b
C. freemani + dough	0.9 b	2.5 b	24.6 a
Dough	0.0 b	0.6 b	0.0 b

[a] Data were combined for the Kilbourne and Washington, Illinois, sites.
[b] In each experiment, means in a column followed by the same letter are not significantly different (LSD, 0.05 level). Analysis was done in log (X + 1) scale, and means were returned to numerical scale for presentation. Sex of captured *C. antiquus* shown for total captures over 10.

TABLE 4. ASSOCIATION BETWEEN *C. antiquus* AND *C. lugubris* IN A SAMPLE OF 120 CORN EARS FROM GREEN VALLEY, ILLINOIS: NUMBERS OF EARS ON WHICH JUST ONE, BOTH, OR NEITHER SPECIES WAS PRESENT

		C. lugubris		Totals for *C. antiquus*[a]
		Present	Absent	
C. antiquus	Present	25 (13.5)[b]	2 (13.5)	27
	Absent	35 (46.5)	58 (46.5)	93
Totals for *C. lugubris*[a]		60	60	120

[a] Overall, 23% of the ears were infested with *C. antiquus*, and 50% were infested with *C. lugubris*. Mean numbers of beetles per ear (standard deviation): *C. antiquus*, 0.44 (0.98); *C. lugubris*, 2.39 (3.61).
[b] Expected values are given in parentheses for numbers of ears with one, both, or neither species under the null hypothesis that the species acted independently. Null hypothesis was rejected (chi-square statistic = 25.3, 1 *df*, $P < 0.001$). Of the 27 ears infested with *C. antiquus*, 25 (93%) also had *C. lugubris*. This was far higher than the 13.5 (50%) expected under the null hypothesis, given the overall abundance of *C. lugubris* in the sample.

The mean number of *C. antiquus* per ear was 0.44 (±0.98 SD); the mean for *C. lugubris* was 2.39 (±3.61 SD).

DISCUSSION

Aggregation Pheromone. As with the other, previously studied *Carpophilus* species (Bartelt et al., 1990a,b, 1991), *C. antiquus* males produce an aggregation pheromone to which both sexes are attracted. As with the other species, the pheromone is synergized by host volatiles, the combination of food- and beetle-derived volatiles being more attractive than either odor source alone.

C. antiquus was unusual, however, in that the pheromone was quite attractive in the field even without a synergist. Only with *C. freemani* was there a comparably high response to the pheromone alone relative to food volatiles (Bartelt et al., 1990b). This property may correlate to an ability to colonize relatively unripe, undamaged fruits (although the beetles are certainly not restricted to these). Male *C. antiquus* or *C. freemani* at such a site could attract conspecifics even before the rich volatile bouquet associated with overripe fruit and accompanying microorganisms is present to synergize the pheromone. Smilanick (1979) found that *C. freemani* tended to accept unripe figs more readily than did *C. mutilatus* or *C. hemipterus*.

In the laboratory, the decreased pheromone-emission rates in the presence of other beetles may be evidence for an aggregation-terminating mechanism. So far we have found no evidence for antiaggregation pheromones in the nitidulids, but a mechanism for terminating aggregations under field conditions must exist.

The relatively low pheromone production per male from groups of beetles had not been noted before in our nitidulid studies, but the phenomenon has been reported in other beetle groups. For example, Burkholder and Dicke (1966) found that groups of 10 or 20 *Trogoderma inclusum* females were far less attractive than groups of two or four females. It was later learned that crowding interfered with calling behavior, resulting in reduced total pheromone emission (W.E. Burkholder, personal communication).

Chemistry. The pheromone of *C. antiquus* (compound **1**) has a novel structure. It is like several other *Carpophilus* pheromones in being an all-*E* tetraene (Bartelt et al., 1992), but it has the most carbon atoms (17) of any so far encountered. It is the first shown unequivocally to have two ethyl side chains. The mass and NMR spectral data are presented for the compound, and a synthetic method for preparing it is outlined. The compound fits the general biosynthetic scheme for the *Carpophilus* pheromones (Bartelt et al., 1992; Petroski et al., 1993); it is probably assembled from two propionate and three butyrate acyl units.

Interactions involving C. freemani *in Field Studies.* One potential compli-

cation in understanding the field studies is that triene **8** was unavoidably produced as an impurity in the synthesis of **1.** Triene **8** was discovered previously as a very minor male-specific hydrocarbon in *C. freemani* (Bartelt et al., 1991b). The compound had significant wind-tunnel activity but is not added to the pheromone for this species for field use because it did not synergize the activity of **7** (Bartelt et al., 1991b). The slight attraction of *C. freemani* to the synthetic pheromone for *C. antiquus* (Tables 2 and 3) was probably due to triene **8,** although the activity of **1** cannot be totally ruled out. In any case, the response of *C. freemani* to the synthetic *C. antiquus* pheromone was far weaker than to its own pheromone (Table 3).

There were also weak trends in Table 3 for cross-attraction between *C. freemani* and *C. lugubris*, but the effects were not significant and were very minor compared with responses to the species' own pheromones. Cross-attraction would not have been surprising because tetraene **5,** the major pheromone component of *C. lugubris*, is also a minor component for *C. freemani*, but the effect was not strong.

Interactions between C. antiquus *and* C. lugubris *in Field Studies.* Although both species responded best to their own pheromones, the responsiveness of *C. antiquus* to the pheromone of *C. lugubris* (Table 3) suggests a kairomonal role for the *C. lugubris* pheromone. *C. antiquus* could locate and cocolonize sites first established by *C. lugubris*. For example, corn ears are an acceptable food source for both species, but *C. lugubris*, being larger and stronger, could more easily penetrate the husk and gain access to and colonize developing kernels. It would be an advantage for *C. antiquus* to locate the *C. lugubris* infestations; otherwise, it could be very difficult for *C. antiquus* to use this food source. The present corn ear sample was collected from an open husk variety, which *C. antiquus* could enter fairly easily, but the association with *C. lugubris* was clear nevertheless, supporting that *C. antiquus* used the *C. lugubris* pheromone to find the ears. Competition from *C. antiquus* on a corn ear probably has little impact on *C. lugubris* because food is rarely limiting even when both species are present.

As with the bark beetles, which have been studied in far greater depth over the years (e.g., review by Birch, 1984), the nitidulid beetles seem to be involved in a variety of chemical interactions under field conditions that go beyond simple pheromonal attraction.

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